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(54) Production of hydrolysate

(57) A process for the production of a hydrolysate which comprises fermenting a protein containing material and a carbohydrate to form a Koji, hydrolysing the fermented Koji at a temperature between 2°C and 50°C and a pH of from 5.6 to 7.0 for a period of from 1 to 20 days characterised in that inoculation with a culture of L.sake at an inoculation density of from 10^3 to 10^7 cfu/g of fermented Koji is carried out before or at the beginning of the fermentation stage. The hydrolysate has a meaty taste and may be used as a liquid sauce, a paste or as a dried powder as a base for an aromatisation agent in culinary products.

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Description

[0001] The present invention relates to a process for the production of a hydrolysate, more particularly to the production of hydrolysate by the biological hydrolysis of protein containing material.

[0002] Hydrolysed proteins have been known for use as seasonings in food systems for centuries in the Far East in the form of soya sauce which traditionally has been prepared by fermentation for a long period of time, usually several months. In producing soya sauce, plant protein containing materials such as cooked soya beans or defatted soy grits together with carbohydrates are inoculated with Aspergilli and the semi solid product is fermented for 2 days to make Koji during which time enzymes are produced which are able to hydrolyse protein and carbohydrates in the moromi stage. The fermented Koji is mixed with a solution of common salt to give moromi which is fermented for 4 to 8 months by the soya lactic acid bacteria and soya yeasts from which the soya sauce is obtained by removing the insoluble fractions from the fermented moromi.

[0003] About 100 years ago, a more rapid method of hydrolysing proteins for producing seasonings was developed using hydrochloric acid in which the time required is only a few hours. However, in recent years, the use of acid hydrolysed plant protein (HPP) in culinary applications has been under criticism due to the presence of chloro-compounds which arises from the acid process. Therefore, attempts have been made to develop HPP replacements which can be used as bodygivers in culinary applications. Soya sauce is one such suitable replacement. However, owing to the differences in the raw materials and the processing methods involved, the two products, HPP and soya sauce, have some differences in terms of chemical composition and flavour profile. Dosage of soya sauce which can be used as an HPP replacement is limited due to its "fermented" note. The different processing procedures also result in a significant variation in the degree of hydrolysis of the protein containing material to the amino acids. Soya sauce has a lower amino acid content than HPP and this leads to a significantly weaker body in soya sauce than in HPP.

[0004] In our co-pending EP-A-96202309.9, we describe a process for the production of a seasoning which comprises preparing a fermented protein koji from a protein containing material and a carbohydrate, hydrolysing the fermented protein koji at a temperature between 15°C and 60°C and a pH of from 4.5 to 10 for a period of from 6 hours to 28 days characterised in that inoculation with a culture of a lactic acid bacteria at an inoculation density of from 10^3 to 10^7 cfu/g of fermented protein koji is carried out either in the fermented protein koji stage or in the hydrolysis stage.

[0005] We have now found surprisingly that if the lactic acid bacteria is Lactobacillus sake (L.sake) and that inoculation is carried out at the beginning of the fermentation process, a hydrolysate is produced which has a very much stronger meaty note than hydrolysates produced using other lactic acid bacteria or when inoculating with L.sake after the fermentation stage. Although not wishing to be bound by theory, we believe that during fermentation, L.sake produces specific enzymes which convert sulphur containing amino acid of the protein substrate, e.g. cysteine into hydrogen sulphide which acts as a precursor for the building blocks for meaty note development.

[0006] Accordingly, the present invention provides a process for the production of a hydrolysate which comprises fermenting a protein containing material and a carbohydrate to form a Koji, hydrolysing the fermented Koji at a temperature between 2°C and 50°C and a pH of from 5.6 to 7.0 for a period of from 1 to 20 days characterised in that inoculation with a culture of L.sake at an inoculation density of from 10^3 to 10^7 cfu/g of fermented Koji is carried out at the beginning of the fermentation stage.

[0007] During hydrolysis, it should be understood that generally, longer periods of time are required at lower temperatures and vice versa.

[0008] The fermented Koji is prepared by a process similar to the conventional soya sauce process which comprises, for example, inoculating a protein containing material and a carbohydrate with, in addition to a culture of L.sake, a culture of Aspergillus oryzae and/or Aspergillus sojae on a culture bed to form the fermented Koji. The L.sake may be added before or after the culture of Aspergillus but when added after the culture of Aspergillus, the addition of the L.sake should take place soon after the commencement of fermentation. L. sake could also be added soon after the commencement of fermentation, e.g. within 1 hour, preferably within 30 minutes, more preferably within 15 minutes, even more preferably within 5 minutes, and especially within 1 minute. The protein containing material is advantageously a plant protein material containing a high proportion of cysteine, e.g. from 0.5 to 3% and preferably from 0.75 to 2% by weight, for instance, soya, wheat germ, corn gluten or rice gluten but is preferably wheat gluten, to produce an increased level of sulphide in the hydrolysate and thus an increased meaty note. The degree of meaty note may also be varied by using a Koji substrate with different proportions of protein and carbohydrate, e.g. a substrate containing from 30 to 100% of protein, preferably from 70 to 90% of protein. The plant protein containing material is advantageously cooked and is preferably used in solid particulate form for enabling the mould of Aspergillus oryzae and/or Aspergillus sojae to grow on the surface of the particles and eventually penetrate into the particles. The particles of the plant protein material, which may be in the form of pellets, preferably have an average diameter of from 2 to 10mm, preferably from 3 to 8mm and especially from 4 to 7mm. The Koji is conveniently fermented in the solid state.

[0009] During the fermentation stage, the L.sake grows rapidly and dominates the flora at a level of 10^8 to 10^9cfu/g at the end of the fermentation, together with A.oryzae. The quality of the Koji is hence clean with respect to other possible contaminants. By adding the L.sake at the beginning of the fermentation, the control of the microflora is controlled right from the start without giving any chance for the contaminants to multiply. The high level of bacteria in the fermented Koji increases the protection against the growth of undesirable microorganisms in the subsequent hydrolysis.

[0010] The hydrolysis of the fermented protein koji in the presence of water may be carried out in the absence or presence of salt and advantageously with constant agitation. Preferably, the hydrolysis is carried out in the absence of salt at a temperature from 30° to 37°C over a period of from 2-5 days in order to maximise the enzyme activity of L.sake, which leads to the production of a stronger meaty flavour in the hydrolysate. When salt is present, the amount may be up to 100% by weight based on the weight of the fermented Koji.

[0011] If desired, additional protein containing a high proportion of cysteine, e.g. wheat gluten, may be added at the beginning of hydrolysis to increase the meaty note.

[0012] At the end of the hydrolysis, the level of the reducing sugar is very low, usually less than 0.3% and therefore, moromi maturation can be eliminated. As a result of this, the whole production time may be shortened by from 1 to 6 weeks.

[0013] After the hydrolysis, the hydrolysed fermented koji together with the culture of L.sake may be pressed to separate a liquid sauce from a solid residue. The liquid sauce is advantageously pasteurised e.g. at a temperature of from 90° to 140°C for a period of from 15 seconds to 30 minutes and then filtered to give a liquid seasoning. If desired, salt may be added either before or after pressing or filtering to give a product having a salt content of 0-60% by weight based on the weight of dried matter. If desired, the liquid sauce may be made into a powder for instance, by concentration, then dried, e.g. vacuum dried to a low moisture content and finally milled into a powder to give a solid seasoning.

[0014] The hydrolysate produced by the process of the present invention may be used as a base, in either liquid?, paste or solid form, for an aromatisation agent for culinary products. A paste may be produced having a dry matter content of from 35 to 55% by weight which comprises drying the hydrolysate and mixing with water, salt, reducing sugar and optionally a sulphur containing amino acid or thiamine, to give a paste containing, on a dry matter basis, from 24 to 97% of the hydrolysate, 2 to 40% salt, 1 to 4% reducing sugar and from 0 to 2% of a sulphur containing compound, e.g. an amino acid such as cysteine or thiamine. If desired, this paste may be heated at 80 to 150°C, preferably from 90 to 110°C for from 1 minute to 4 hours, preferably from 1 to 2 hours. The heated paste may afterwards be dried to a residual water level of up to 2%.

[0015] The hydrolysates of the present invention may be used in process flavour applications, e.g. beef and chicken, body givers for culinary products and as ingredients for HPP replacers. When the sauce powder is reconstituted in water, the product has a much lighter colour, a strong meaty flavour and is more neutral with respect to the typical fermented note found in the standard wheat gluten sauce.

[0016] The present invention will now be further illustrated by the following Examples in which parts and percentages are given by weight.

Example 1

[0017] A mixture of wheat gluten and wheat bran (93:7) was extruded through a Clextral extruder to obtain pellets having an average diameter of 5mm with a porous structure.

[0018] 75 kg of the extrudates were mixed with 25kg of roasted wheat and soaked in 75 kg water at 75°C for 5 minutes. The soaked extrudates were then heated to 100°C and held at the same temperature for 10 minutes and afterwards cooled to below 40°C by applying vacuum. The cooked extrudates were mixed with a liquid suspension of 25g of Aspergillus oryzae spores inoculum followed by 340g of a culture of L.sake at 7 x 10^5cfu/g of cooked extrudates.

[0019] During the 42 hours of koji fermentation, the following temperature profiles were maintained for the culture bed:

0 - 25 hours	30°C
25 - 42 hours	27°C

[0020] The koji was mixed at 18^{th} and 25^{th} hours to ensure sufficient airflow through the culture bed for good ventilation. The microbiological quality was very good during the fermentation where the coliform count was $<10^2$cfu/g throughout. After the fermentation, the level of L.sake had risen to 3.3 x 10^9cfu/g.

[0021] After the Koji fermentation, the matured Koji was harvested. Hydrolysis was carried out in a hydrolysis tank by adding water to obtain a hydrolysate with a total solid content of 20.2m/m. The hydrolysis was carried out at 35°C for 48 hours.

[0022] During the hydrolysis, a rapid pH drop (initial pH=6.4) was observed due to the growth of the L.sake. A pH of 6.0 was reached after approximately 2 hours of hydrolysis. Thereafter, a 40% NaOH solution was dosed in to maintain the pH at 6.0. The high count of L.sake observed in the Koji continued to dominate in the hydrolysate and the development of coliforms was again under good control.

[0023] Finally, the hydrolysed mixture was pressed to separate a wheat gluten sauce from a solid residue. Salt was added to a level of 12%m/m before pressing. The wheat gluten sauce was treated at 90°C for 20 minutes.

The liquid sauce was concentrated by evaporation. The concentrate obtained was dried in a vacuum oven and then milled into a powder.

[0024] For organoleptic evaluation, 12.5g of liquid sauce or 3.5 g powder were diluted with 250 ml of boiling water. In both cases the product was found to have a meaty note background which does not have the disadvantage of the "fermented note". The product was very light in colour and was versatile in culinary applications.

Example 2

[0025] A similar procedure to that described in Example 1 was followed except that the koji prepared according to Example 1 was mixed with wheat gluten powder (Koji:wheat gluten powder=7:3) in the hydrolysis. The water quantity was adjusted such that the dried matter content of the hydrolysate was 20.2%.

[0026] A higher level of sulphide was detected in the hydrolysate compared to Example 1. This is due to a higher protein content (TN=13.0%dried weight basis) compared to wheat gluten koji alone (TN=11.3%dried weight basis), which gave rise to a higher content of cysteine. The reduced carbohydrate content also reduced the availability of carbohydrate source for the metabolism of the L.sake. As a result, there was a higher uptake of cysteine and production of hydrogen sulphide. The hydrolysate had a strong meaty flavour.

Example 3

[0027] A similar procedure to that described in Example 1 was followed except that the carbohydrate content was reduced in the substrate for Koji preparation. The mixture used was 85% wheat gluten, 5% of wheat bran and 10% of wheat flour. L.sake and A.oryzae were inoculated in the cooked substrate. The microbiological results showed that growth of L.sake was not affected by the change to reduced carbohydrate recipe. A higher level of sulphide was detected in the hydrolysate compared to Example 2 and the hydrolysate had a strong meaty flavour.

Example 4

[0028] A similar procedure to that described in Example 3 was followed except that salt was not added in the hydrolysate, and a salt free sauce or powder was obtained with a strong meaty flavour.

Example 5

[0029] A similar procedure to that described in Example 1 was followed except that the hydrolysis was carried out at 22°C for 10 days. A salt free sauce or powder was obtained with a strong meaty flavour.

Example 6

[0030] A similar procedure to that described in Example 3 was followed except that the hydrolysis was extended to 5 days to increase the degree of hydrolysis. A salt free sauce or powder was obtained with a strong meaty flavour.

Example 7

[0031] A similar procedure to that described in Example 2 was followed except that corn gluten powder was used instead of wheat gluten powder. A salt free sauce or powder was obtained with a strong meaty flavour.

Example 8

[0032] The dried hydrolysate, described in Example 1 was used as base for an aromatisation agent for culinary products. For the preparation of this agent, 47.8 parts of the hydrolysate powder was mixed with 17.0 parts of water, 13.3 parts of salt, 8.3 parts of yeast extract, 1.1 parts of cysteine, 1.1 parts of thiamine, 0.8 parts of glucose and 0.1 parts of onion extract. The paste was heated in a double jacketed kettle for about 90 minutes at 100°C and dried under a reduced pressure of 15mbar to a dry matter level of 98%.

Comparative Example

[0033] A similar procedure to that described in Example 1 was followed except that pH of the hydrolysis was kept floating, i.e. no pH adjustment. The pH of the hydrolysate dropped to 4.5 after 4 hours of hydrolysis. After 8 hours of hydrolysis, the pH was adjusted to 6 and maintained throughout the 48 hours of hydrolysis. With this pH profile, the level of sulphide was negligible throughout the 48 hours of hydrolysis. The corresponding sauce had a less meaty note than the product of Example 1.

[0034] These results indicate that the pH should be greater than 5.6 at least during the growth phase of L.sake (about 2-8 hours hydrolysis.

Claims

1. A process for the production of a hydrolysate which comprises fermenting a protein containing material and a carbohydrate to form a Koji, hydrolysing the fermented Koji at a temperature between 2°C and 50°C and a pH of from 5.6 to 7.0 for a period of from 1 to 20 days characterised in that inoculation with a culture of L.sake at an inoculation density of from 10^3 to 10^7 cfu/g of fermented Koji is carried out at the beginning of the fermentation stage.

2. A process according to claim 1 wherein the fermented Koji is prepared by inoculating a protein

containing material and a carbohydrate with, in addition to a culture of L.sake, a culture of Aspergillus oryzae and/or Aspergillus sojae on a culture bed to form the fermented Koji.

3. A process according to claim 1 wherein the protein containing material is a plant protein material containing a high proportion of cysteine.

4. A process according to claim 1 wherein the protein containing material is soya, wheat germ, corn gluten, rice gluten or wheat gluten

5. A process according to claim 1 wherein the protein containing material is a substrate containing from 30 to 100% of protein.

6. A process according to claim 1 wherein the protein containing material contains from 70 to 90% of protein.

7. A process according to claim 1 wherein the protein containing material is cooked and is used in solid particulate form for enabling the mould of Aspergillus oryzae and/or Aspergillus sojae to grow on the surface of the particles and eventually penetrate into the particles.

8. A process according to claim 1 wherein the protein containing material is fermented in the solid state.

9. A process according to claim 1 wherein the hydrolysis of the fermented protein koji in the presence of water is carried out in the absence or presence of salt.

10. A process according to claim 1 wherein the hydrolysis is carried out in the absence of salt at a temperature from 30° to 37°C over a period of from 2-5 days.

11. A process according to claim 1 wherein when salt is present, the amount is up to 100% by weight based on the weight of the fermented Koji.

12. A process according to claim 1 wherein additional protein containing a high proportion of cysteine is added at the beginning of hydrolysis.

13. A process according to claim 1 wherein, after the hydrolysis, the hydrolysed fermented koji together with the culture of L.sake is pressed to separate a liquid sauce from a solid residue.

14. A process according to claim 13 wherein the liquid sauce is pasteurised at a temperature of from 90° to 140°C for a period of from 15 seconds to 30 minutes and then filtered to give a liquid seasoning.

15. A process according to claim 13 wherein salt is added either before or after pressing to give a product having a salt content of 0-60% by weight based on the weight of dried matter.

16. A process according to claim 13 wherein the liquid sauce is made into a powder by concentration, then dried to a low moisture content and finally milled into a powder to give a solid seasoning.

17. A process for the preparation of a paste having a dry matter content of from 35 to 55% by weight for use as a base for an aromatisation agent in culinary products which comprises drying the hydrolysate prepared by the process of claim 1 and mixing with water, salt, reducing sugar and optionally a sulphur containing amino acid or thiamine, to give a paste containing, on a dry matter basis, from 24 to 97% of the hydrolysate, 2 to 40% salt, 1 to 4% reducing sugar and from 0 to 2% of a sulphur containing amino acid or thiamine.

18. A process according to claim 17 wherein the paste is heated at 80 to 150°C for from 1 minute to 4 hours.

19. A process for the preparation of a dry hydrolysate for use as a base for an aromatisation agent in culinary products which comprises drying the paste obtained as in claim 18 to a residual water level of up to 2%.

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EUROPEAN SEARCH REPORT

Application Number
EP 97 11 9011

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	US 4 308 284 A (FUMIO NODA, K.;ET AL) * claim 1 * ---	1	A23L1/23 A23L1/328 A23L1/20
Y	EP 0 417 481 A (SOCIETE DES PRODUITS NESTLE S.A.) * column 3, line 25-35; claim 1 * ---	1	
Y	DATABASE WPI Section Ch, Week 9343 Derwent Publications Ltd., London, GB; Class D13, AN 93-338881 XP002023697 & JP 05 244 897 A (SAITAMA KEN) , 24 September 1993 * abstract * ---	1	
A	US 4 587 127 A (TAKESHI AKAO;ET AL) * the whole document * -----	1,2,4,7, 17	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6) A23L

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	26 February 1998	Caturla Vicente, V

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
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